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Research Article

Evaluation of non-opioid analgesic toxicity and their effects on native and invasive species of crustaceans (Gammaridae, *Gammarus*)

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Abstract

Humans widely use pharmaceuticals due to their availability, effectiveness, quick pain relief, and ability to improve well-being and health conditions. Their presence in aquatic environments, even in small doses, leads to serious ecological disturbances and alterations in trophic chains. This study aimed to assess the mortality and mobility of native *Gammarus pulex* and invasive *Gammarus tigrinus* under exposure to the nonopioid analgesics paracetamol, ibuprofen, ketoprofen, and a pharmaceutical mixture, as well as to determine their acute toxicity and impact on both species. At the 24th and 96th h of exposure, the highest increase in mortality was observed, most likely linked to shock caused by the sudden appearance of pollutants and the duration of exposure. Mortality was influenced not only by exposure time but also by the type of pharmaceutical. Paracetamol was the most lethal for both native and invasive species, though 100% mortality was recorded after 96 h of exposure. At a concentration of 5000 mg/L, all organisms died within 24 h. *G. tigrinus*, despite its broader ecological tolerance to pollution and resistance to environmental changes, responded to the presence of pharmaceuticals like *G. pulex*. Regarding survival, the ibuprofen–ketoprofen mixture in a 1:1 ratio was the least lethal for *G. tigrinus*, while ketoprofen alone was the least lethal for *G. pulex*. The availability and widespread use of over-the-counter pharmaceuticals, combined with the lack of systematic monitoring of aquatic environments, pose serious challenges to aquatic organisms in the context of pharmaceutical pollution in water habitats.

Key words: Gammarids, nonopioid drugs, time exposition, toxicity, water pollution

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Introduction

Pharmaceuticals are widely used by humans due to their common availability, effectiveness, quick pain relief, and their role in improving well-being, health conditions, and supporting the immune system. Analgesics, anti-inflammatory drugs, antipyretics, and dietary supplements are commonly available not only in pharmacies but also in grocery stores, supermarkets, and cosmetic drugstores, which often leads to their abuse and improper use (Mondal et al. 2025; Samsul et al. 2025). In Poland, compared to other European countries, pharmaceutical consumption is relatively high and continues to increase (Drobniewska et al. 2024). The widespread use of pharmaceuticals results in their intentional or

accidental release into the natural environment (Zuccato et al. 2006; Daughton 2008; Aib et al. 2025).

The presence of pharmaceuticals in aquatic ecosystems has been discussed at both global and European forums for many years (European Environmental Agency 2010; European Commission 2010). Currently, the associated risk is classified as relatively low, but with recommendations to monitor the vulnerable population, expand knowledge of long-term exposure effects, and prioritize risk assessment for selected substances. Nevertheless, scientists warn that in the near future, pharmaceuticals in aquatic ecosystems may pose indirect or direct threats not only to aquatic organisms but also to humans, as their concentrations continue to rise (Costa et al. 2023; de Souza Neri et al. 2025).

The chemical and metabolic stability of some pharmaceuticals means that up to 90% of the active ingredient may be excreted in its original form. In aquatic environments, pharmaceuticals undergo processes that reduce their concentrations, but the rate and extent vary depending on the substance. Pharmaceuticals occur in water in dissolved or colloidal form. Once released into water, they are absorbed into bottom sediments and may also be biodegraded by living organisms. They can bind to suspended particles, be transported over long distances, and decompose under the influence of abiotic factors (Stamm et al. 2008; Kucharski et al. 2022). Sediments have a high capacity to degrade organic compounds due to abundant bacterial activity; however, the rate of decomposition depends on oxygen availability and is slower in deeper anaerobic layers (Warren et al. 2003). Due to their high polarity, low volatility, and lipophilicity (Skrzypczak 2012), pharmaceuticals are readily transported in aquatic environments, bioaccumulate in tissues, and biomagnify through food chains (Warren et al. 2003; Santos et al. 2010). This biomagnification may also affect humans who consume fish and other aquatic organisms that accumulate drugs in their tissues (Ochoa-Esteso et al. 2024; Satyaprakash et al. 2025).

As part of water monitoring under the Water Framework Directive (WFD 2000; Directive 2000/60/EC) — the overarching European Union legislation adopted in 2000 to establish a common framework for the protection and management of inland surface waters, transitional waters, coastal waters, and groundwater, with the objective of achieving “good ecological and chemical status” — only a limited number of pharmaceuticals are regularly controlled. In its strategic approach to pharmaceuticals in aquatic environments, the European Commission highlighted the narrow scope of monitoring, focusing on “hot spots,” such as sewage discharge from hospitals (European Commission 2020). The potential cumulative effects of multiple pharmaceuticals remain poorly understood; however, their combined presence can cause a range of negative outcomes in aquatic organisms (Aib et al. 2025). These effects occur at multiple levels of biological organization. At the organism level, exposure to pharmaceuticals can induce behavioral changes (Aich et al. 2025), disrupt key biochemical pathways, and interfere with hormonal, immunological, and metabolic processes. Such disturbances may impair survival and, in severe cases, lead to organism mortality. At the developmental level, pharmaceuticals can adversely affect growth and differentiation, while also disrupting reproductive processes. These impacts may reduce reproductive success and compromise early life stages, ultimately leading to population decline. At the ecosystem level, the cumulative

consequences of these effects can contribute to ecological imbalance. In addition, pharmaceutical residues and their transformation products may deteriorate water quality — for example, by influencing oxygen availability and other physicochemical parameters — thereby exacerbating ecological stress (Larsson 2014). Taken together, these impacts pose risks not only to aquatic ecosystems but also to public health, underscoring the need for measures to mitigate the presence and effects of pharmaceuticals in aquatic environments (Küster and Adler 2014; Boroń and Pawlas 2015; Eapen et al. 2024).

Gammarids are commonly used in ecotoxicological studies to monitor water pollution, particularly *Gammarus pulex*. Its resistance to pollution depends on the type and concentration of pollutants present in the environment (Shahid et al. 2018; Khattab et al. 2022). *G. pulex* is highly sensitive to chemical toxicity, physical changes in water parameters, alterations in water quality, pesticide contamination, heavy metals, surfactants (e.g., detergents), as well as fluctuations in temperature and oxygen content (Russo et al. 2018). For this reason, it serves as a reliable bioindicator of water pollution, with its presence or absence reflecting the degree of contamination. In contrast, invasive gammarid species such as *Gammarus tigrinus* often exhibit greater tolerance to environmental stressors and pollutants (Gruszka 1999). Their broader ecological plasticity and adaptive capacity may enable them to persist in degraded habitats where native species decline. Comparing native and invasive Gammarus species therefore provides valuable insight into differential sensitivity to contaminants and the potential ecological consequences of biological invasions.

The European Commission has emphasized that knowledge about pharmaceuticals released into the environment remains insufficient (European Commission 2010; European Commission 2019). Most pharmaceuticals have not been adequately tested for environmental or human and animal risks. Previous studies have examined the effects of pharmaceuticals on *G. pulex*, including changes in ventilation and locomotion (Lange et al. 2009), responses to low concentrations of fluoxetine and ibuprofen (De Lange et al. 2006), metabolomics (Gómez-Canela et al. 2016), and the influence of ibuprofen and paracetamol (Rigal 2018). However, there is no comparable data for invasive species. Studies on the impact of nonopioid analgesics on alien and invasive Gammarus species have not yet been conducted, and their effects remain undetermined for both alien and native species. Therefore, the present study aimed to assess the mortality and mobility of native and invasive Gammarus species under exposure to selected nonopioid analgesics, and to compare the responses of *G. pulex* and *G. tigrinus* to commercially available pharmaceuticals. The study aimed to determine the acute toxicity (LC50 and EC50 indices) of paracetamol, ibuprofen, ketoprofen, and their mixture, and to conduct a comparative analysis of their effects on both species. We hypothesized that the pharmaceutical mixture would exhibit greater toxicity than individual compounds due to potential additive or synergistic interactions. Furthermore, we hypothesized that *Gammarus tigrinus*, as an alien species, would demonstrate higher tolerance to pharmaceutical exposure than the native species, potentially reflecting differences in ecological adaptability and resistance. This assumption is based on the generally higher ecological plasticity and stress tolerance observed in many invasive taxa, which may enable them to cope more effectively with chemically altered or degraded environments.

Materials and methods

Location of the tested gammarid sites of occurrence

The test organisms were collected from two aquatic environments. The native *G. pulex* was obtained from the water reservoir located in the Natural Monument "Wywierzyska Strzemieszyce," which originated after mineral excavation. The spring water supplies two ponds, which serve as a habitat for species that are found only in pure calcium-magnesium waters, including *Crenobia alpina*, *Bythinella austriaca*, and *Salmo trutta m. fario*. The invasive *G. tigrinus* was sampled from the Kłodnica River, a right-bank tributary of the Oder River, 84 km in length, with a total catchment area of 1125.8 km^2.

The Kłodnica River is considered one of the most heavily transformed riverbeds in the Silesian Upland of southern Poland due to long-term and intensive coal mining. Mining activity led to the creation of mining settlements and significant riverbed modifications, including regulation, shortening of its course, embankment, and concreting (Czaja 1999; Kupka and Szczypek 2021). The river receives large amounts of sewage, mainly via tributaries, as well as domestic and industrial wastewater and mine water.

Economic activity and urban development strongly affect the physical and chemical parameters of the Kłodnica River. Elevated levels of nitrogen and phosphorus compounds, excessive salinity, and high concentrations of total suspended solids are commonly recorded. In addition, the heavy metal content in Kłodnica bottom sediments is considerably higher than in rivers from Polish regions less affected by anthropogenic pressure (Nocoń 2006; Nocoń et al. 2006).

Laboratory analyses and exposure to pharmaceuticals

Gammarids were collected from the bottom sediments of the spring and the river using a hydrobiological net and transported to the laboratory in 5-L containers filled with water from their respective collection sites. In the laboratory, specimens of both species were acclimated for 24 h in site-specific water at a standardized temperature of 12 ± 1 °C, with oxygen saturation (≥90%) maintained using an aeration pump. The use of water from the collection sites was intended to minimize handling stress and abrupt physicochemical changes; however, potential differences in water quality between sites may represent a confounding factor when interpreting interspecific sensitivity. A photoperiod of 16 h light/8 h dark was provided. During the experiment, gammarids were kept in glass laboratory containers with varying concentrations of pharmaceuticals (Table 1). Water collected from the sampling sites was used in the experiment to minimize potential shock from sudden environmental changes and to reduce premature mortality.

At the same time, analyses of selected physicochemical parameters of water from the Strzemieszyce spring and the Kłodnica River were conducted to assess environmental differences and the potential exposure of native and invasive Gammarus species to pollution. Water samples were transported to the laboratory in a cooler and analyzed immediately after the field sampling. In situ, the following parameters were measured, e.g., Conductivity, TDS and pH using the portable HI 9811–5 pH/EC/TDS/°C meter (Hanna Instruments, USA). Other physico-chemical parameters were analyzed in the Hydrobiological Laboratory

Table 1. The set-up of experimental studies.

Solution symbol	Water [ml]	Concentration of pharmaceutical [mg]	Concentration of pharmaceuticals in the exposition [mg/l]
C1 initial solution	100	500	5000
C2	90	50	500
C3	90	5	50
C4	90	0.5	5
C5	90	0.05	0.5
C6 control exposition	100	0	0

of the Institute of Biology, Biotechnology, and Environmental Protection, Faculty of Natural Sciences, the University of Silesia, using the reagents from Merck and Hanna Instruments.

The following active pharmaceutical substances were used in the exposure experiments: paracetamol (500 mg), ibuprofen (500 mg), ketoprofen (500 mg), and a mixture containing 250 mg of ibuprofen and 250 mg of ketoprofen. Five concentrations were prepared for each pharmaceutical (Table 1), using water from the natural habitats of each species. A drug mixture at a 1:1 ibuprofen-to-ketoprofen ratio was also prepared to compare its effects with those of the individual pharmaceuticals. A control sample, consisting of water from the reservoir and the river, served as the reference for organisms not exposed to drugs. Control samples were maintained under the same conditions as the experimental treatments.

Drug solutions were placed in glass containers, and the gammarids were exposed to them for specified durations. Survival and motility of gammarids, as well as changes in behavior under exposure to nonopioid pharmaceuticals, were assessed at 24, 48, 72, and 96 h. Organisms that did not respond to the touch of a laboratory needle were considered dead and were removed from the experimental solutions.

Calculation of indices

The LC50 value, representing the concentration required to kill 50% of the tested organisms within a given time period, and the EC50 value, representing the concentration required to immobilize 50% of the organisms, were determined using probit analysis (Zhang et al. 2007). LC50 values were measured at 24, 48, 72, and 96 h of exposure and are expressed in mg/L. EC50 values were calculated based on the proportion of organisms showing immobilization during continuous exposure to the tested substances. For both LC50 and EC50, confidence intervals were estimated using standard statistical methods.

Results

The physicochemical parameters of the water from which gammarids were sampled showed clear differences (Table 2). Conductivity, TDS, ammonia, and nitrite levels were higher in the Kłodnica River, the habitat of *G. tigrinus*. Only pH values showed no significant differences between the sampling sites (Table 2). While these parameters differ between sites, it should be noted that *G. tigrinus* naturally thrives under the higher conductivity and nutrient conditions observed

Table 2. The physicochemical parameters of the water from habitats of species occurrence were tested.

Parameter	The Strzemieszyce source – *G. pulex* habitat	The Kłodnica River – *G. tigrinus* habitat
pH	7.7	7.9
Conductivity (µS cm^{-1})	839	8230
TDS (mg dm^{-3})	461	4520
Ammonium nitrogen (mg dm^{-3})	0	9.1
Ammonia NH_4 (mg dm^{-3})	0	11.06
Nitrate nitrogen (mg dm^{-3})	1.6	0
Nitrates NO_3 (mg dm^{-3})	7.088	0
Nitrite nitrogen (mg dm^{-3})	0.046	0.145
Nitrites NO_2 (mg dm^{-3})	0.151	0.477
Phosphates (mg dm^{-3})	0.04	1.21
Salinity (‰)	0.03	4.6

in the Kłodnica River, suggesting that these differences are unlikely to have imposed additional stress on this species. Nevertheless, the influence of varying water characteristics on experimental outcomes should be acknowledged as a potential, albeit minor, factor in interpreting mortality patterns.

LC50 and EC50 indicators

According to the assumption of acute toxicity, the longer the exposure of organisms to a reagent, the lower the concentration required to cause mortality (LC50) or immobilization (EC50). The reliability of the confidence range (+/−) increases the closer it is to the baseline value. Single LC50 and EC50 results with very high deviations may indicate either drug concentrations too low to kill 50% of the organisms (values unreadable by the program) or inconsistent results without a clear trend (Table 3).

LC50 and EC50 values showed high variability and wide confidence intervals, which limited their interpretative power. In several cases, toxicity thresholds could not be reliably determined, which suggest either insufficient concentrations to elicit a 50% response or inconsistent organismal responses without a clear time-dependent trend. Consequently, a consistent decline in LC50 or EC50 values with increasing exposure time was not observed, contrary to expectations for acute toxicity testing (Table 3).

Analysis of the toxicity of the studied non-opioid drugs

Paracetamol (A)

For all pharmaceuticals tested, mortality generally increased with concentration and duration of exposure in the case of both Gammarus species; however, responses varied significantly between drugs and between native and invasive gammarids (Fig. 1). Paracetamol showed a clear time- and concentration-dependent effect in both species of crustaceans. In the first 24 hours, mortality was limited to the highest concentration, with *G. tigrinus* responding faster and more strongly than the native species. In the following days, mortality gradually appeared also at lower concentrations, with the toxicity threshold initially being higher for the invasive species.

Table 3. Values of LC50 and EC50 for Gammarus species in the time exposition on non-opioid analgesic.

	Gammarus pulex				*Gammarus tigrinus*			
	A Paracetamol	B Ibuprofen	C Ketoprofen	D Mixture	A Paracetamol	B Ibuprofen	C Ketoprofen	D Mixture
LC 50 [mg/L]								
24 h	-	-	-	68.030 +/- 739.094	-	-	-	-
48 h	-	-	-	2.935 +/- 7.525	-	3633.233 +/- 68982.729	-	-
72 h	-	-	-	43.550 +/- 717.02	-	-	-	-
96 h	-	0.185 +/- 1.258	-	-	-	-	-	-
EC50 [mg/L]								
24 h	-	-	-	-	-	-	-	-
48 h	6617.616 +/- 559385.22	134.782 +/- 600.228	-	-	172.275 +/- 851.439	1347.819 +/- 6812.515	-	102.436 +/- 306.251
72 h	5.0 +/- 15.79	363.323 +/- 6162.672	-	-	-	-	-	-
96 h	-	0.185 +/- 1.258	-	-	-	-	-	-

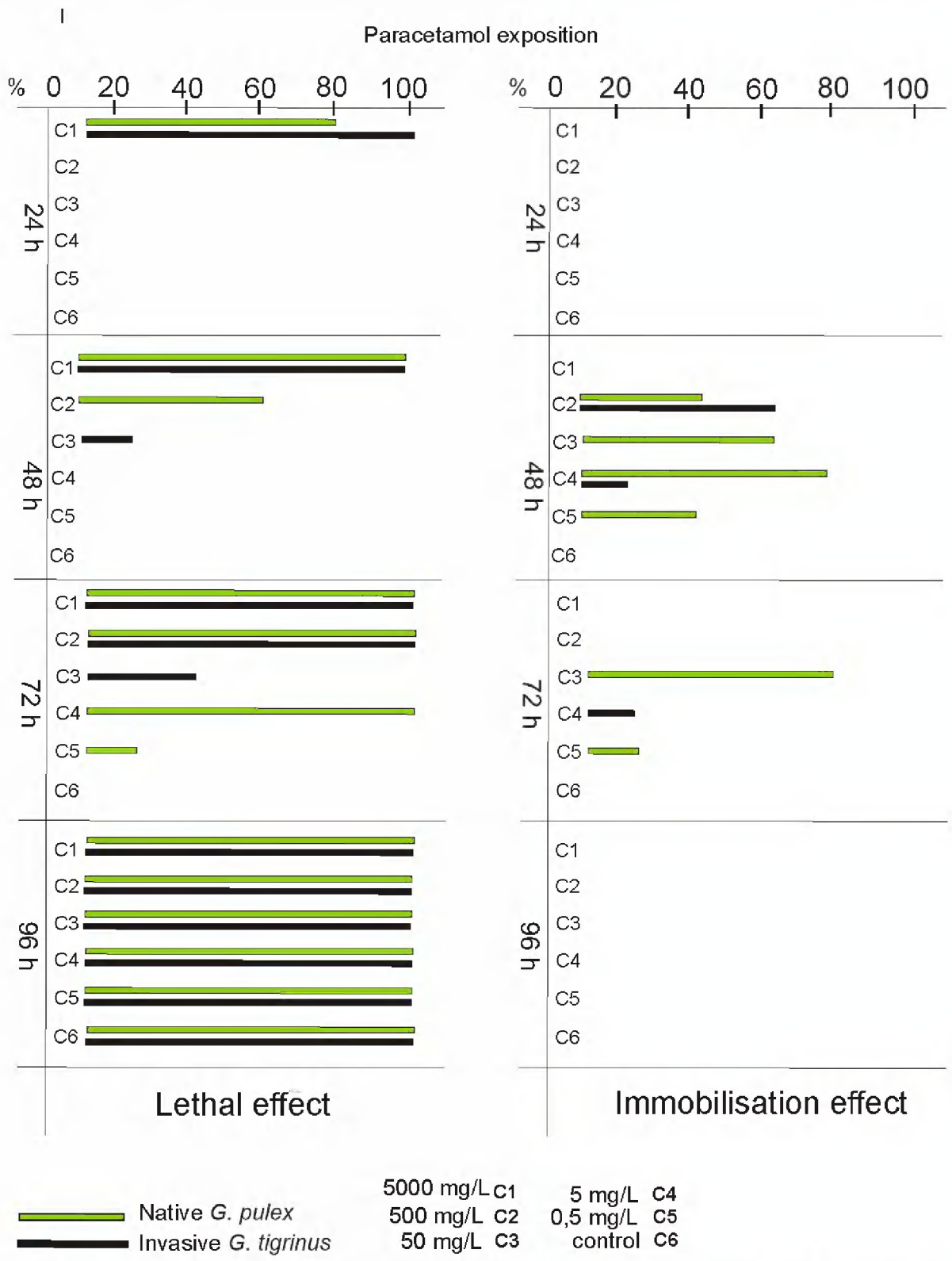

Figure 1. Lethal and immobilizing effect of paracetamol on native *G. pulex* and invasive *G. tigrinus* in the time of exposure.

After 72 hours, mortality increased dramatically, affecting both native and invasive species, and the differences between them became blurred. Finally, after 96 hours of exposure, paracetamol proved lethal to all individuals at all tested concentrations, while control individuals survived, confirming the compound's potent toxicity (Fig. 1).

Gammarids' immobilization did not occur in the initial phase of the experiment and reached a maximum after 48 hours of exposure, especially in *G. pulex*. This phenomenon was strongly concentration-dependent but did not constitute a definite stage preceding death, suggesting that paralysis may have been a transient stress response rather than a direct indicator of lethality (Fig. 1).

Ibuprofenum (B)

Ibuprofen exhibited a strong and rapid toxic effect at the highest concentration, causing complete mortality in both species within the first 24 hours. At lower concentrations, responses were more variable and clearly species-dependent. *G. pulex* generally showed higher sensitivity in the early stages of exposure, while in *G. tigrinus*, mortality increased more slowly and was delayed (Fig. 2).

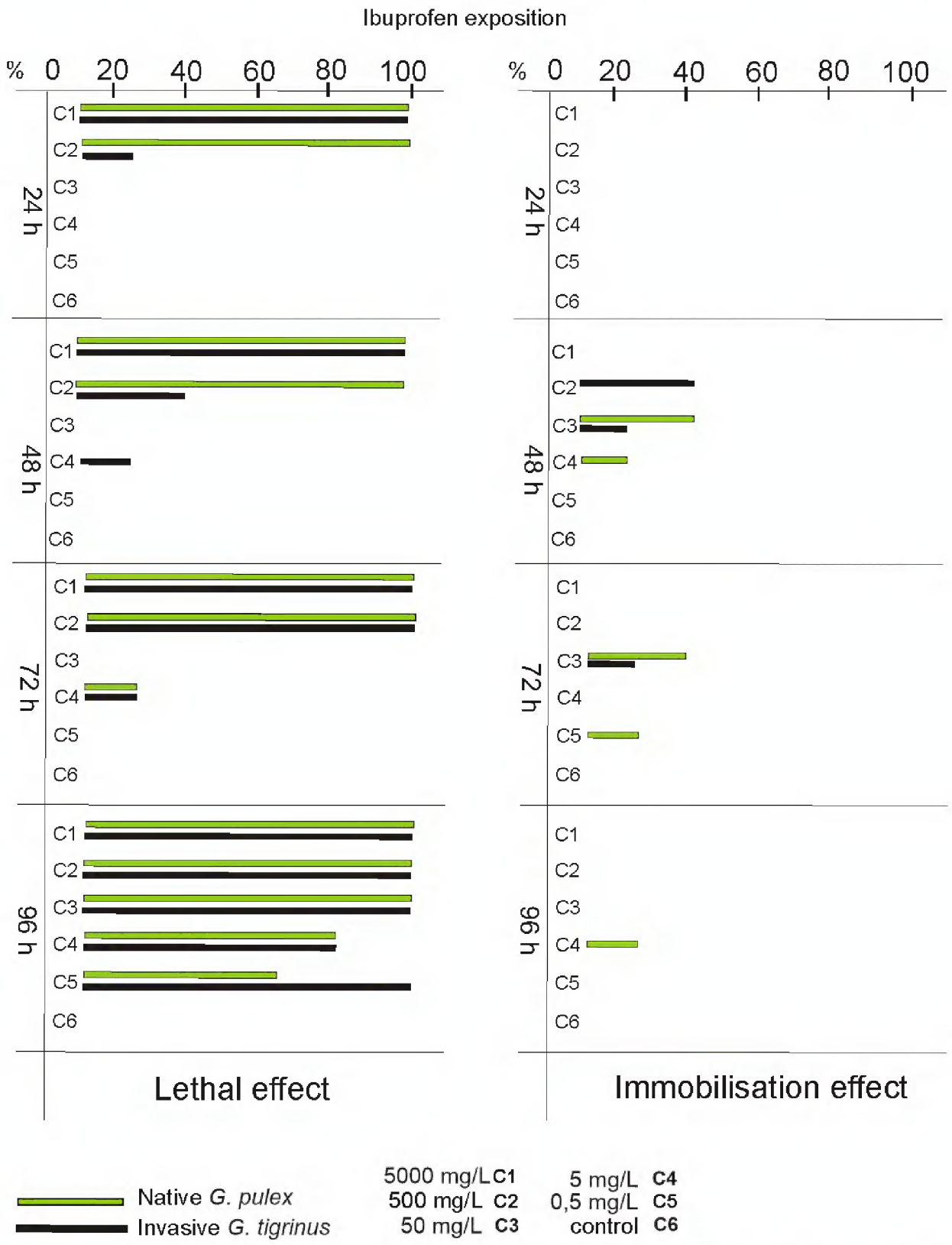

Figure 2. The lethal and immobilizing effect of ibuprofen on native *G. pulex* and invasive *G. tigrinus* in the time of exposure.

With increasing exposure time, the differences between species gradually decreased, and after 96 hours, a rapid increase in mortality was observed, even at concentrations that had previously elicited no toxic effects (Fig. 2). This indicates the cumulative nature of ibuprofen's effects.

Immobilization occurred primarily after 48 hours of exposure and was transient in nature – many individuals previously considered immobilized were classified as dead on the following days. The lack of a clear "paralysis – death" sequence suggests that immobilization was not a stable or predictable indicator of ibuprofen toxicity (Fig. 2).

Ketoprofenum (C)

Ketoprofen caused high mortality in both species even at the highest concentration on the first 24 hours, but at intermediate concentrations, its effect was more gradual and variable. *G. pulex* showed an earlier lethal response at moderate concentrations, while in *G. tigrinus*, mortality increased more slowly but reached high levels by the final phase of the experiment (Fig. 3).

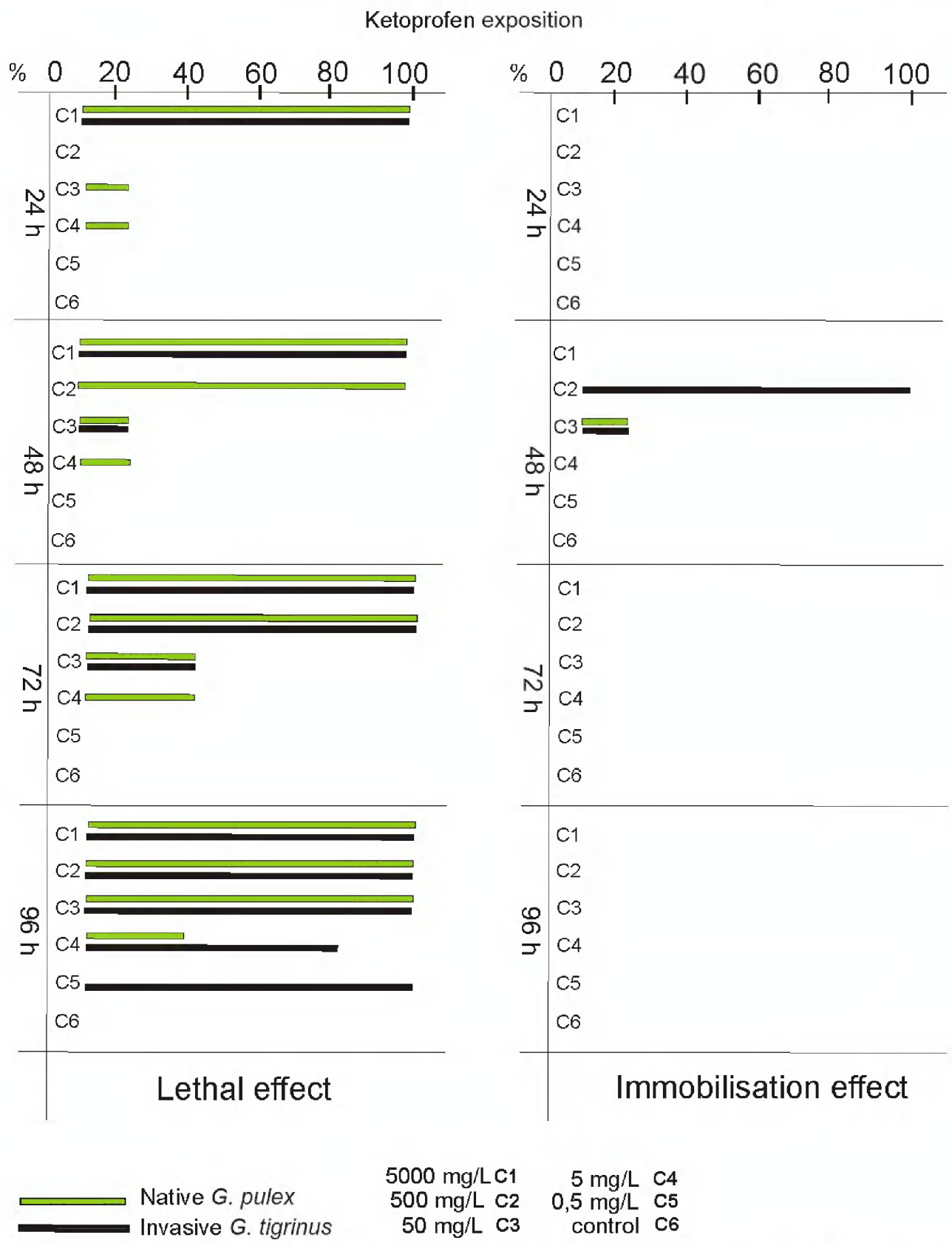

Figure 3. The lethal and immobilizing effect of ketoprofen on native *G. pulex* and invasive *G. tigrinus* in the time of exposure.

After 72 and 96 hours, there was a significant increase in mortality in the invasive species, particularly at lower concentrations, blurring the initial differences between the species. Ultimately, both species showed comparable susceptibility to ketoprofen, although the dynamics of the response were different (Fig. 3).

Immobilization occurred sporadically and only after 48 hours of exposure, indicating that ketoprofen acted primarily through direct lethality rather than through a prolonged sublethal phase (Fig. 3).

Mixture of Ibuprofenum i Ketoprofenum (D)

The ibuprofen and ketoprofen mixture was characterized by a strong toxic effect, causing complete mortality in both species within the first 24 hours at the highest concentration. At lower concentrations, the organism's response was more complex and clearly dependent on the species and exposure time (Fig. 4).

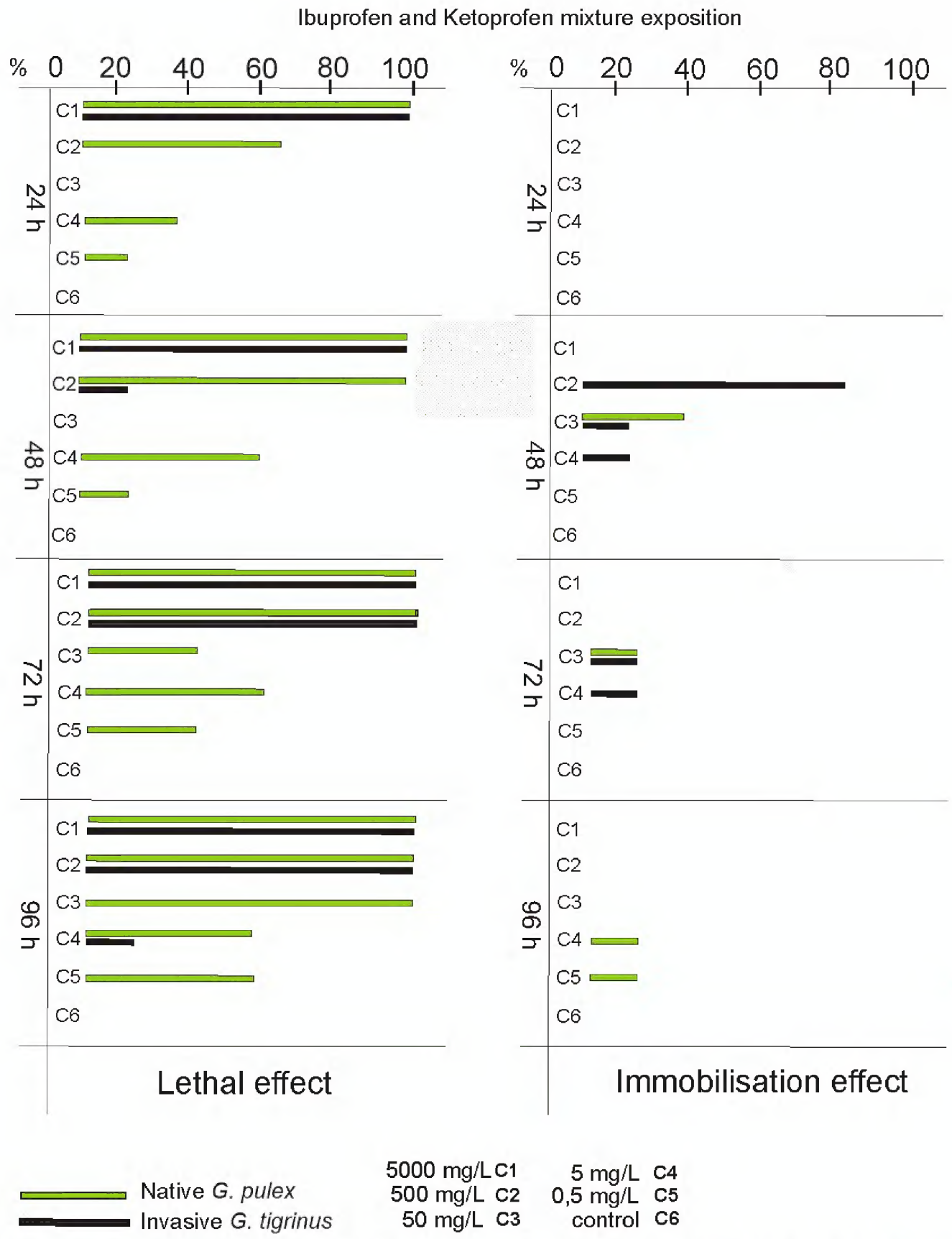

Figure 4. The lethal and immobilizing effect of the Ibuprofen and ketoprofen mixture on native *G. pulex* and invasive *G. tigrinus* in the time of exposure.

G. pulex showed earlier and more pronounced mortality at intermediate concentrations, while *G. tigrinus* showed a delayed but rapid response leading to complete mortality in the following days (Fig. 4). This indicates the lack of a simple, additive toxic effect of the mixture and possible interactions between the substances.

Immobilization increased after 48 hours of exposure, however, similarly as in the case with individual substances, it was primarily transient and often preceded death (Fig. 4). The variability of the response and the lack of clear patterns underscore the complex nature of the effects of pharmaceutical mixtures on aquatic organisms.

Discussion

Pharmaceuticals, as a relatively new threat to aquatic ecosystems, increasingly require study to better understand their impact on organisms and water quality, particularly in the context of pharmacovigilance by European Union Member States. Nonopioid drugs represent the first step of the analgesic ladder, meaning they are used to relieve mild to moderate pain of various origins, such as headache, muscle pain, nerve pain, or post-traumatic pain (Hawkey and Lanas 2001). Our study represents the first comparative assessment of the acute toxicity of paracetamol, ibuprofen, ketoprofen and their mixture in native (*Gammarus pulex*) and invasive (*Gammarus tigrinus*) ampipods. The results clearly show that the reactions of the organisms depended on the pharmaceutical compound, its concentration, exposure time, and species identification, highlighting the complexity of pharmaceutical toxicity in aquatic invertebrates. Although the physicochemical properties of water differed between sampling sites, *G. tigrinus* is an introduced species well adapted to conditions in the Kłodnica River, where it occurs at high densities. Therefore, these environmental differences are unlikely to have imposed significant additional stress on this species. Nevertheless, variations in water properties should be acknowledged as a potential factor in interpreting sensitivity to tested substances, particularly when considering other, more sensitive species. It should be noted that the use of site-specific water for each species may have influenced the observed responses, as differences in physicochemical parameters could partly account for variation in toxicity endpoints. Therefore, the results should be interpreted with caution, and future studies should consider conducting experiments under fully standardized water conditions to disentangle species-specific sensitivity from environmental effects. Pharmaceutical toxicity studies are increasingly important in the era of anthropogenic change, as they help reveal the potential ecological impacts of substances that may act as significant pollutants in aquatic environments (Keddy et al. 1995; Fick et al. 2010; Traczewska 2011; Wydro et al. 2016; Cunha et al. 2017). Our findings support the view that pharmaceuticals in aquatic environments, even at low doses, can cause serious ecological disturbances and pose potential risks within aquatic trophic chains. In all treatment groups, mortality generally increased with concentration and exposure time, although the relationship was not always linear. Two clear peaks in mortality were observed, occurring after 24 and 96 hours of exposure. An early peak likely reflects acute stress after sudden exposure to high concentrations, while a later peak suggests accumulation or delayed toxic effects.

Similar temporal relationships have been noted in other studies of drug-exposed aquatic invertebrates (Gerhardt et al. 2005; Rigal 2018).

Aquatic organisms are exposed to the simultaneous action of multiple pollutants, leading to chemical interactions that can modify the active compounds to which organisms are exposed. Such interactions may result in potentiation, where the combined action of factors produces effects greater than the sum of their individual impacts. These processes contribute to an increase in the biological toxicity of substances (Traczewska 2011; Grzesiuk et al. 2018). Although the toxicity of individual or combined pharmaceuticals may be low, even trace concentrations (a few mg/L) can cause mortality or functional disorders in aquatic organisms. In this study Paracetamol proved to be the most toxic for both species, ultimately causing 100% mortality at all concentrations after 96 hours. Although early mortality was limited to the highest concentration, toxicity quickly spread to lower concentrations with longer exposure. This indicates that paracetamol exerts delayed but severe effects, which is consistent with reports that sub-lethal exposure may initially cause impairment of function before leading to irreversible damage (Gerhardt et al. 2005; Rigal 2018). Importantly, despite initial differences in susceptibility, both *G. pulex* and *G. tigrinus* were moving towards total mortality, suggesting limited, long-term, species-specific tolerance to the compound.

Ibuprofen and ketoprofen showed more heterogeneous toxicity patterns. At the highest concentrations, both drugs caused rapid and complete mortality within 24 hours, confirming their strong acute toxicity. However, at intermediate concentrations, the reactions differed between species. *G. pulex* generally showed earlier mortality, while *G. tigrinus* often showed a delayed response, particularly to ibuprofen. These results suggest temporary tolerance of invasive species to moderate pharmaceutical stress, although this advantage declined with increasing exposure time. Marchlewicz et al. (2015) observed significant changes in the life activity of *G. pulex* exposed to low doses of ibuprofen (0.1–10 μg/m^3). At 1 μg/m^3, a concentration commonly detected in surface waters, a 35% decrease in life activity was recorded. Notably, a study conducted in 20 countries worldwide reported the presence of ibuprofen in over 90% of analyzed samples, with wastewater concentrations reaching 3.35 μg/dm^3. In river waters near Madrid, ibuprofen concentrations reached 5 μg/dm^3, while ketoprofen was measured at 1.56 μg/dm^3 (Matejczyk 2022).

In the natural environment, pharmaceuticals most often occur as mixtures rather than single compounds (Wiącek-Rosińska 2008; Anderson and Lydy 2002). Research by Adamek et al. (2011) showed that mixtures of drugs with other medicinal substances at high concentrations are more toxic to aquatic organisms such as *Daphnia magna*, leading to immediate death. A combined solution of acetylsalicylic acid, ibuprofen, naproxen, and diclofenac exhibited much stronger toxicity compared to each of these drugs tested separately. Similarly, a mixture of fluoxetine and clofibric acid caused 50% mortality of *D. magna* under laboratory conditions after 144 h of exposure, while mixtures of fluoxetine, ibuprofen, and ciprofloxacin increased fish mortality (Szymonik and Lach 2012). Long-term exposure of organisms to low concentrations of drugs mixtures often results in harmful physiological effects at the initial stage, followed by death (Pascoe et al. 2003). In our study the pharmaceutical mixture (ibuprofen + ketoprofen, 1:1) did not produce a direct additive effect. While the

highest concentration was immediately lethal to both species, lower concentrations resulted in species-specific and time-dependent responses. Importantly, *G. tigrinus* showed higher survival than G. pulex at a few intermediate concentrations, indicating greater resistance to combined exposure. This finding partially supports the hypothesis that invasive species with wider ecological tolerance can better tolerate complex chemical stressors. However, this resistance was not universal and did not prevent high mortality with long-term exposure, highlighting the non-linear nature of the mixture's toxicity. Immobilization reactions further illustrate this complexity. Paralysis was most often observed after 48 hours of exposure and did not always precede death. In many cases, the immobilized individuals either temporarily recovered or were later classified as dead. This suggests that immobilization represents a reversible response to stress rather than a reliable predictor of mortality, which has also been observed in previous ecotoxicological studies (Pascoe et al. 2003).

Contrary to expectations, *G. tigrinus* did not consistently show higher drug resistance than *G. pulex*. Although invasive species showed delayed mortality or greater tolerance under certain conditions – particularly to ibuprofen and a pharmaceutical mixture – these differences were dependent on the chemical compound and duration of action. Ultimately, prolonged exposure and higher concentrations eliminated most of the interspecies differences, indicating that invasive status alone does not confer widespread resistance to pharmaceutical contaminants. Overall, our findings show that the toxicity of non-opioid analgesics to aquatic invertebrates is highly specific to the chemical compound and depends on both exposure dynamics and species characteristics. Paracetamol posed the greatest risk to both species, while ibuprofen and ketoprofen produced more variable and species-dependent effects. The reactions observed underline the need for a multi-species, exposure time and pharmaceutical mixture toxicity assessment to better reflect real-world conditions in aquatic ecosystems.

Although the Water Framework Directive (OJ L 327, 22.12.2000) obliges EU Members to jointly apply water quality and pollutant emission standards, illegal discharges into aquatic systems still occur. In 2013, the European Parliament approved Directive 2013/39/EU, amending Directives 2000/60/EC and 2008/105/EC regarding priority substances, and introduced an obligation to monitor chemical compounds from the so-called "watch list" in surface waters. Among the substances included were pharmaceuticals such as diclofenac and estriol (Kuczyńska 2017). Systematic monitoring, testing, and control of pharmaceutical residues in aquatic environments are crucial for developing effective strategies to neutralize and prevent environmental damage. Research on pharmaceutical toxicity to aquatic organisms is indispensable (Szymonik and Lach 2012).

Conclusion

This study demonstrates that non-opioid anti-inflammatory drugs can exert potent, compound-specific effects on both native and invasive gammarid species, and toxicity depends on exposure time, drug type, and species. Contrary to initial expectations, invasive *G. tigrinus* did not consistently show higher resistance to pharmaceutical stress than native *G. pulex*, indicating that invasive status alone does not guarantee increased tolerance to drug contamination. The results indicate that paracetamol is a particularly dangerous compound for

aquatic invertebrates, while ibuprofen and ketoprofen produced more variable and context-dependent effects, especially when used in a mixture. The lack of simple additive reactions to combination drugs highlights the complexity of the toxicity of mixtures and the limitations of risk assessment for individual compounds. Overall, our findings highlight that pharmaceutical contamination poses a heterogeneous but potentially serious threat to freshwater invertebrates, and species-specific responses can alter the structure of communities under conditions of chronic or repeated exposure. These results confirm the need for multi-species ecotoxicological studies and realistic exposure scenarios to improve the assessment of the environmental risks posed by pharmaceutical products in aquatic ecosystems. From these findings alone, we can conclude that the availability and widespread use of over-the-counter pharmaceuticals, combined with the lack of systematic monitoring of aquatic environments, pose serious challenges to aquatic organisms in the context of pharmaceutical pollution in water habitats.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Conceptualization AC, BO; Methodology AC, BO, KC, KR Software AC; Validation AC, RC; Formal analysis AC; Data curation AC; Writing – original draft, AC, AS; Writing – review & editing AC, Visualization, AC; All authors have read and agreed to the published version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the article.

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